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(54) Title: FLAVOUR DELIVERING SYSTEMS COMPRISING A MICROEMULSION OR HYDRATED REVERSED MICELLES

(57) Abstract

Flavour releasing compositions comprise water in oil microemulsion droplets and/or hydrated reverse micelles. The cores may contain a flavour precursor and an enzyme; an active flavour is produced by the action of the enzyme.

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# FLAVOUR DELIVERING SYSTEMS COMPRISING A MICROEMULSION OR HYDRATED REVERSED MICELLES

The present invention relates to flavour-releasing compositions comprising water-in-oil microemulsion droplets and/or hydrated reverse micelles, to food-grade microemulsions and hydrated reverse micelles suitable for use as flavour delivery agents in foods and in the mouth, and to processes, compositions and reactors for the enzymic synthesis of various flavours, fragrances and flavour/fragrance precursors in vitro.

## Introduction

### Food flavour

The flavour of foods is important to both consumers and the food processing industries as the demand for food quality increases. There is therefore great interest in flavours which can be added to food products to enhance or modify the flavour and/or aroma characteristics of the food, and much research has been carried out into the nature of the chemicals responsible for producing various flavours and aromas, as well as into the way in which these chemicals trigger sensory responses before and during eating.

It is now recognized that flavour and aroma perception arises from a chain of events: mixtures of chemicals (flavours) are liberated from food as it is eaten; these chemicals then interact with sensors in the mouth and nose; finally, the signals from the sensors are processed in the brain to give the sensation of flavour and/or aroma. The sensations of flavour and aroma are therefore closely linked, and the term flavour is used herein to include aromas.

As a result of this research, it is now recognized that in order to obtain good flavour characteristics it is essential not only to have the right types of chemicals present in foods, but also to deliver them to the sensors at the appropriate rate. The initial burst of flavour when foods are first bitten, as well as the lingering aftertaste of other foods, are phenomena which arise from the differential release of flavour compounds over time.

However, attempts to enhance flavour by supplementing foods with flavour compounds have been disappointing. In general, flavour compounds are unstable, particularly in the environment of the food, and break down into flavourless degradation products. Attention has therefore focused on systems for stabilizing flavour compounds in foods.

While such attempts to stabilize and then deliver flavours from encapsulated systems have proved successful in preventing degradation of the flavour compounds prior to consumption, it has generally been found that the release characteristics in the mouth do not always match the desired release profile. Moreover, this problem is exacerbated by the fact that mastication of food typically occurs over a period of 30 seconds, so that rapid release is crucial to flavour quality. However, encapsulated systems generally require longer time periods for full flavour release, so that they are unsuitable for applications in which an initial burst of flavour generated on chewing is desirable.

There is therefore a need for flavour delivery systems in which the flavour elements remain stable during storage, but which release flavour rapidly when required (e.g. during eating).

Over the past few years, it has been shown that many flavours exist as flavour precursors which are more stable than the active flavour itself. For example, many flavours (especially in plant foods) exist as glycoside precursors which are much more stable than the flavour aglycone, but possess no flavour properties. When food is macerated, either during food preparation or in the mouth during mastication, glycosidase enzymes act on the glycosides so generating flavour molecules.

It has now been recognized that flavour delivery systems based on the use of stable flavour precursors which are activated during eating (or shortly before) obviates the need for stabilized flavours, so overcoming the problems associated with poor flavour delivery from such stabilized systems. In particular, the present inventors have found that *microemulsion technology* can be exploited to stably maintain flavour precursors prior to eating while permitting rapid delivery of flavour moieties derived from the precursors in the mouth (or shortly before eating).

Microemulsions

Microemulsions (MEMs) are systems of oil, water and surfactant which exist as single phase liquid solutions that are optically isotropic and thermodynamically stable (Danielsson and Lindmann (1981); *Colloids Surfaces* 3, pp. 391 *et seq.*).

Microemulsions therefore differ fundamentally from *emulsions*, which are simply droplet dispersions of one liquid in another (oil in water or water in oil). As such, emulsions are thermodynamically *unstable* and ultimately separate into distinct oil and water phases as droplet coalescence and coagulation occurs.

In some instances, the term "microemulsion" has also been used by those skilled in the art to define compositions comprising very small droplets in a medium, the droplets usually having diameters in the nm size range (and so also referred to as "nanodroplets" or "nanoemulsions").

As used herein, the term "microemulsion" is intended to embrace compositions falling within the scope of both of the definitions set out above.

The structure, properties and known uses of microemulsions is reviewed in Rees and Robinson (1993); *Adv. Mater.* 5(9), pp. 608-619, the disclosure of which relating to the structure of microemulsions and organogels is incorporated herein by reference.

The extent and position of the single phase microemulsion region for a typical surfactant (or combination of surfactants) is shown in the schematic phase diagram (Figure 1).

For water-in-oil (w/o) microemulsions, the parameter $R$ and the surfactant concentration define the composition. $R$ is the mole ratio of water to surfactant (Eq. 1).

$$R = [H_2O]/[\text{surfactant}] \qquad (1)$$

At low $R$ values ($< 10$) in w/o microemulsions, the droplets are relatively small and the amount of water present is only just sufficient to fully hydrate the surfactant head groups and counterions. Under these circumstances, the aggregates are generally described as *hydrated reverse micelles*. Where there is enough water present in the droplet or reverse micelle cores to satisfy or exceed the hydration requirements of the surfactant, then so-called "free" water may be present and at this point the aggregates are generally referred to as water-in-oil microemulsion droplets ($R$ = 10 - 20). At $R$ values > 20, droplet sizes are larger and the water inside the w/o microemulsion droplets behaves as bulk water in terms of its gross physical properties.

Microemulsions in the form of droplets are essentially monodisperse, the droplets having radii within the range 1-100 nm (more usually, 1-10 nm).

Microemulsion surfactants and co-surfactants

Microemulsion formation is dependent on the presence of amphiphilic surfactant molecules having both polar and non-polar regions which stabilize the oil-water

interface. In many formulations, co-surfactants are also required to act as spacers and maintain the spacing between charged headgroups (so minimizing coulombic repulsions).

The manner in which surfactants pack at the interface is primarily dependent on steric effects arising from the configuration of the surfactant molecule. Packing behaviour has been described mathematically by Mitchell and Ninham (1981); *J. Chem. Soc., Faraday Trans. II*, 77, page 601). Packing is described by the packing parameter $S_p$ (Eq. 2):

$$S_p = V(al)^{-1} \qquad (2)$$

where $V$ is the effective packing volume of the surfactant hydrocarbon tail, $l$ is the length of the hydrocarbon tail and $a$ is the effective headgroup area at the oil-water interface.

For oil-in-water microemulsion droplets, $S_p < 1$; for lamellar structures, where the interface is flexible and exhibits both negative and positive curvature, $S_p$ is about 1; for reverse micelles and water-in-oil emulsions, $S_p > 1$.

Preparation of microemulsions

The preparation of microemulsions is technically trivial and the preparation of many different microemulsion compositions has been described in detail in the prior art. Typically, the surfactant is first dissolved in the oil and then water is added with gentle shaking. This procedure generally results in the formation of an optically-clear single-phase solution containing microemulsion droplets. Heating or sonication (e.g. in sonicating water baths) is not necessary to achieve the single-phase dispersion, though such steps may be employed where convenient to facilitate preparation.

Multiphase microemulsion-containing systems

Such systems were first described by Winsor (1948); *Trans. Faraday Soc.*, 44: page 376. Here, an oil-in-water microemulsion may co-exist with an oil phase (Winsor I), or a water-in-oil microemulsion may co-exist with excess water (Winsor II). Also possible is a Winsor III system, which forms when the surfactant is concentrated in a surfactant-rich middle phase which co-exists with oil and water phases containing low concentrations of droplets (water-in-oil and oil-in-water, respectively).

Winsor I to Winsor II interconversion can be induced by changing the temperature or ionic strength of the microemulsion composition or through the addition of co-surfactants. The ability to effect such interconversions has great utility in microemulsion-based synthetic systems, since phase interconversion provides a simple means of separating water-soluble products from surfactant (Winsor II) as well as oil-soluble species from surfactant (Winsor I).

Microemulsion solubilization of enzymes

It is known that enzymes can be solubilized in the droplet cores of w/o microemulsion droplets with retention of activity and stability. A large number of enzymes have been solubilized in this way (see Rees and Robinson, *infra*). Although the resultant systems are essentially a single phase, they can assimilate both water-insoluble and water-soluble substrates.

In microemulsion systems where the enzymic reaction consumes water, enzyme activity ceases as water content decreases. This happens quite rapidly in systems containing nanometre droplets with limited water content in the droplet cores. Experiments with lipase have shown that activity in a MEM system halts when the water is consumed, but if more water is added the reaction restarts and continues until the additional water is used up. This cycle of activity can be repeated many times with full activity returning on the addition of water.

Microemulsion systems containing solubilized enzymes have also been used in organic synthesis, and syntheses on the preparative scale have been reported with hydrolases such as chymotrypsin and lipase (see Rees and Robinson, *op cit*). These synthetic systems have been used to produce esters from alcoholic and fatty acid precursors for use in the food industry (West (1988); *Chem. Br.* (Dec): p. 1220).

It is also known that the equilibrium position of MEM-solubilized hydrolases can be shifted in favour of amide/ester synthesis, either by exploiting the mass-action effect (when product is rapidly partitioned away from the hydrophilic reaction microenvironment into the non-polar oil phase) and/or the low water activity present in the droplet/micelle core at low $R$ values.

Microemulsion-based organogels

The addition of gelatin to w/o microemulsions may result in the formation of rigid gels of a strength similar to those obtained when gelatin is added to water. While the molecular structure of microemulsion-based organogels (MBGs) has

not yet been fully elucidated, several models have been proposed (see Rees and Robinson, *infra*).

MBGs may be easily prepared by incubating the parent microemulsion at 50°C and mixing with a solution of gelatin in water at the same temperature. The resulting mixture is then shaken vigorously and allowed to cool, whereupon an optically transparent single phase gel is formed. Gel strength is controlled by varying the amount of gelatin present.

Detailed description of the invention

According to the present invention there is provided a flavour releasing composition comprising water-in-oil microemulsion droplets and/or hydrated reverse micelles, wherein the droplet/micelle cores contain a latent flavour.

The latent flavour may comprise a flavour precursor which is activated when the droplet/micelle cores are subjected to the conditions prevailing when they are chewed in the mouth (including for example shear, dilution, hydration, mixing, contact with saliva and with the oral mucosa).

In a preferred embodiment, the latent flavour comprises a flavour precursor which is activated by enzymes present in saliva so that flavour is released when foodstuff containing the flavour releasing composition is eaten.

Preferably, the droplet/micelle cores have low water activity and contain a latent flavour which is activated when the water activity within the cores is increased.

Alternatively, the droplet/micelle cores may contain a latent flavour together with an activating enzyme and a cognate enzyme inhibitor. In such embodiments, the activating enzyme catalyses the production of active flavour from the latent flavour substrate only when the cognate inhibitor is diluted out by saliva during mastication of foodstuffs containing the flavour compositions.

In such embodiments, the inhibitor may be a component of the microemulsion system itself (e.g. the surfactant or co-surfactant). For example, propylene glycol may fulfil a dual role, both as a co-surfactant and as an enzyme inhibitor.

Particularly preferred are compositions in which the latent flavour is a system comprising:

(a) a flavour precursor; and

(b) an enzyme which is inactive due to the low water activity within the cores,

wherein the enzyme acts on the flavour precursor to produce an active flavour moiety on hydration of the cores.

Without wishing to be bound by any theory, it is thought that such compositions release flavour upon eating as the temperature and water content (contributed by saliva) increase and shear forces (*via* mastication) are applied. The increase in water content drives the microemulsion through a phase change into a Winsor II multiple emulsion (to form a water-in-oil microemulsion with excess water). This excess water forces a phase separation. The increase in temperature increases the rate of collision between the microdroplets and this, together with the shear forces and increase in water activity, causes swelling and disruption of the microdroplets. Thus, the enzyme present in the cores becomes catalytically active and converts the flavour precursor into active flavour products. The constant shearing and mixing decompartmentalizes the enzyme, substrate and products, so that flavour is released.

The flavour delivery system may also be used to release a cascade of different flavours over time to produce a particular desired flavour profile.

This may be achieved by using combinations of different enzymes and cognate substrates within a single MEM system, or by using mixtures of different MEMs each having different solubilized enzyme/precursor systems. Control over the rate of release may then be achieved through any or a combination of the following mechanisms:

(a) varying the activity of the enzyme (for example, by selecting a particular biological source having a desired activity). Suitable enzyme sources include bacterial, fungal (e.g. yeast), plant, animal (e.g. mammalian) sources, and may include those derived from thermophilic and halophilic organisms); and/or

(b) varying the activity of the enzyme by incorporating specific enzyme inhibitors (e.g. glucose inhibits glucosidases); and/or

(c) varying the enzyme/substrate concentration; and/or

(d) providing one or more of the MEMs in the form of organogels, which slows the rate of delivery of the flavours produced therein; and/or

(e) varying the nature of the precursor (e.g. at the level of the number of glycoside sugar residues); and/or

(f) varying the identity of the surfactant and/or co-surfactant, since some surfactants (e.g. propylene glycol) may act as enzyme inhibitors; and/or

(g) varying the relative proportions of the different MEM components present in a mixed MEM system.

The flavour precursor may comprise any of a large number of known flavour glycosides. The flavour glycoside may be a fruit (e.g. strawberry) flavour or a savoury flavour glycoside (for example a mustard oil glycoside, e.g. a glucosinolate or thioglucoside).

The active flavour moiety is preferably a flavour aglycone. Particularly preferred are flavour aglycones having the general formula R-OH. In such embodiments, R may be selected from an aliphatic alcohol residue, an aromatic alcohol residue (for example a terpene, e.g. menthol, geraniol or citronellol) and an alicyclic alcohol residue. Such embodiments may find particular application in the delivery of sweet, sour and/or fruit flavours.

Alternatively, the flavour aglycone may have the general formula R-SH. Such embodiments find particular application in the delivery of savoury flavours.

The enzyme for use in the invention may be selected from any of oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases or combinations of different enzymes of any of the aforementioned type.

Particularly preferred are hydrolases selected from ester hydrolases, glycosyl hydrolases, ether (e.g. thioether) hydrolases, peptide hydrolases or combinations of any of the foregoing. Such enzymes find particular application in the generation of flavour aglycones from glycoside flavour precursors.

Preferred ester hydrolases for use according to the invention include carboxylic ester hydrolase (e.g. lipase), thioester hydrolase, phosphoric monoester hydrolase, phosphoric diester hydrolase, triphosphoric monoester hydrolase, a sulphuric ester hydrolase, diphosphoric monoester hydrolase and combinations of any of the foregoing.

Preferred glycosyl hydrolases for use according to the invention include those which hydrolyse *O*-glycosyl residues, and in particular $\alpha$-glucosidase, ß-glucosidase, $\alpha$-galactosidase or ß-galactosidase). Other preferred glycosyl hydrolases include those which hydrolyse *N*-glycosyl or *S*-glycosyl compounds. In the latter case, particularly preferred for savoury applications is myrosinase,

which finds particular application in the hydrolysis of mustard oil glycosides (such as glucosinolates and thioglucosides) to aglycones of general formula R-SH).

Preferred peptide hydrolases include $\alpha$-aminoacylpeptide hydrolase, peptidylamino-acid or acylamino-acid hydrolases, dipeptide hydrolases, dipeptidylpeptide hydrolases, peptidyldipeptide hydrolases, proteinases (e.g. serine proteinases, SH-proteinases, acid proteinases or metalloproteinases) or combinations of any of the foregoing.

The microemulsion droplets and/or hydrated reverse micelles for use in the invention are preferably food grade, though in some applications (e.g. petfoods) non-food grade materials may be used provided that the final concentrations are non-toxic.

Thus, particularly preferred for use in the invention are droplets/micelles in which the oil is an animal or vegetable oil. Any of a wide variety of known and readily available animal or vegetable oils may be used, but sunflower oil has been found to be particularly suitable.

The droplets or micelles may further comprise a surfactant, and any surfactant with a low hydrophilic-lipophilic balance value may be used. Examples of suitable surfactants for use in the invention include sorbitan esters (e.g. sorbitan monooleate), glycerol derivatives (e.g. stearyl monoglyceride), phospholipids (e.g. lecithin), naturally occurring phosphoglycerides (e.g. phosphatidylcholine or phosphatidylethanolamine) and mixtures of one or more of these surfactants.

Particularly preferred as a surfactant (especially where sunflower oil is used) is a mixture of lecithin and sorbitan monooleate.

In most microemulsion/micelle formulations a co-surfactant is used in addition to the surfactants discussed above. Any suitable co-surfactant may be used, provided that it functions to stabilize the water-oil interface. Particularly preferred are co-surfactants selected from short chain alcohols, propylene glycol or mixtures thereof.

The surfactant/co-surfactant mix for use in the compositions of the invention are preferably selected such that the packing parameter $S_p$ (as hereinbefore defined) of the surfactant/co-surfactant mix is greater than 1.

Particularly preferred according to the invention are double tail and/or medium

tail length surfactants/co-surfactants. Surfactant/co-surfactants having relatively high *V* to *a* ratios (as hereinbefore defined) have been found to be particularly useful for use in the invention.

In preferred embodiments, the *R* value of the microemulsion droplets and/or hydrated reverse micelles in the compositions of the invention is preferably less than 10 (e.g. less than 5). In such embodiments, the water activity in the droplet/micelle core is very low, and may render the solubilized enzyme(s) latent and/or modify enzyme activity and/or specificity.

The droplet/micelle size is preferably 1-100 nm, and in most applications is 1-10 nm.

In a second aspect of the invention there is provided a composition comprising food grade water-in-oil microemulsion droplets or hydrated reverse micelles.

Such food grade microemulsion compositions have not hitherto been described in the prior art, and absent the recognition of the utility of microemulsion systems in the delivery of food flavours there had been no incentive for those skilled in the art to develop such systems.

In a third aspect, the invention provides a process for the organic synthesis of a flavour or flavour precursor comprising the step of enzymically synthesising the flavour or flavour precursor in a water-in-oil microemulsion.

Preferably, the microemulsion droplet cores contain:

(a) a substrate; and

(b) an enzyme which acts on the substrate to produce the active flavour or flavour precursor.

The enzymic synthesis may be conveniently controlled by varying the *R* value of the microemulsion. In this way, the catalytic activity and/or specificity of the enzyme may be altered.

In a particularly preferred embodiment, the enzyme is a hydrolase and the *R* value is selected to shift the equilibrium position of the enzymic reaction such that the enzyme acts synthetically rather than hydrolytically, for example to synthesise any of:

(a) an ester;

(b) a glycosyl compound;

(c) an ether (e.g. a thioether);

(d) a peptide;
(e) a carbon-nitrogen bond (e.g. in an amide, amidine or nitrile compound);
(f) an acid anhydride (e.g. a phosphoryl- or sulphonyl-containing anhydride);
(g) a carbon-carbon bond (e.g. in a ketone compound); or
(h) a halide, phosphorous-nitrogen, sulphur-nitrogen or carbon-phosphorous bond.

Any convenient method may be used to recover the active flavour or flavour precursor, and the method of choice will depend on the nature of the products (particularly their charge). Those skilled in the art will therefore be able to devise suitable separation protocols using common general knowledge and routine trial and error.

In preferred embodiments, the flavour/flavour precursors are recovered by driving the microemulsion droplets through a phase boundary by subjecting the microemulsion droplets to a temperature change to yield a Winsor I, Winsor II or Winsor III type microemulsion system.

Also contemplated by the invention are active flavours and flavour precursors obtainable by (or produced by) the synthetic processes of the invention.

The invention also contemplates a system for use in the process of the invention, the system comprising a water-in-oil microemulsion, wherein the microemulsion droplet cores contain:

(a) a substrate; and
(b) an enzyme which acts on the substrate to produce the active flavour or flavour precursor.

Also contemplated is a reactor comprising the above-described system.

The catalytic activity of the enzyme may be controlled by varying the *R* value of the microemulsion. In preferred embodiments, the *R* value is selected such that the enzyme acts synthetically rather than hydrolytically, for example to synthesise an ester, a glycosyl compound, an ether (e.g. a thioether), a peptide, a carbon-nitrogen bond (e.g. in an amide, amidine or nitrile compound), an acid anhydride (e.g. a phosphoryl- or sulphonyl-containing anhydride), a carbon-carbon bond (e.g. in a ketone compound) or a halide, phosphorous-nitrogen, sulphur-nitrogen or carbon-phosphorous bond.

In particularly preferred embodiments the system is used to synthesize a flavour aglycone or glycoside precursor.

Also contemplated are foodstuffs comprising the flavour releasing composition of the invention.

Also contemplated by the invention is an active flavour or flavour precursor obtainable by (or produced by) the synthetic processes of the invention.

The invention also contemplates the use of a composition comprising water-in-oil microemulsion droplets and/or hydrated reverse micelles for flavour delivery (e.g. flavour delivery in the mouth).

Foodstuffs for use with the invention

The compositions of the invention find application as flavour delivery agents in any foodstuff in which flavour is desirable. As used herein, the term "foodstuff" includes both solid and liquid foodstuffs, including beverages and yoghurts.

Thus, foodstuffs for use with the compositions of the invention include foods selected from crumb, cottage cheeses, aerosol toppings, frozen yoghurts, milk shakes, ice cream, low calorie products such as dressings and jellies, batters, tarts (e.g. fruit tarts) cake mixes, frozen chips, binders, gravies, pastas, noodles, doughs, pizza toppings, sauces, mayonnaise, jam, preserve, pickles, relish, fruit drinks, syrups, toppings and confectionery (e.g. soft centres), petfood, canning gels, coatings, glazes, baits, binders, meat and meat analogue products (for example vegetarian products), gelatin replacers or dairy products or ingredients (e.g. a yoghurt supplement).

## Examples

The invention will now be described with reference to several examples, which are purely exemplary and not intended to be limiting in any way.

### Example 1: Flavour enhancing composition

The following MEM composition was used to deliver the flavour enhancer furaneol:

| | |
|---|---|
| vegetable oil | 80.5% |

| | |
|---|---|
| water | 3.0% |
| lecithin | 15.0% |
| furaneol glycoside | 1.0% |
| glycosidase | 0.5% |

Other formulations with lower levels (as low as 0.02% furaneol) were also produced.

CLAIMS:

1. A flavour releasing composition comprising water-in-oil microemulsion droplets and/or hydrated reverse micelles, wherein the droplet/micelle cores contain a latent flavour.

2. The composition of claim 1 wherein the droplet/micelle cores have low water activity and contain a latent flavour which is activated when the water activity within the cores is increased.

3. The composition of claim 1 wherein the latent flavour is a flavour precursor and the droplet/micelle cores further contain an enzyme and an inhibitor thereof, wherein in use the inhibitor is diluted out so activating the enzyme which acts on the flavour precursor to produce the flavour.

4. The composition of claim 2 wherein the latent flavour is a system comprising:

(a) a flavour precursor; and

(b) an enzyme which is inactive due to the low water activity within the cores,

wherein the enzyme acts on the flavour precursor to produce an active flavour moiety on hydration of the cores.

5. The composition of claim 4 wherein the flavour precursor comprises a flavour glycoside (for example a mustard oil glycoside, e.g. a glucosinolate or thioglucoside).

6. The composition of claim 4 or claim 5 wherein the active flavour moiety is a flavour aglycone.

7. The composition of claim 6 wherein the flavour aglycone has the general formula: R-OH.

8. The composition of claim 7 wherein R is selected from:

(a) an aliphatic alcohol residue;

(b) an aromatic alcohol residue (for example a terpene, e.g. menthol, geraniol or citronellol);

(c) an alicyclic alcohol residue.

9. The composition of claim 6 wherein the flavour aglycone has the general formula: R-SH.

10. The composition of any one of claims 4-9 wherein the enzyme is selected from any of:

(a) *oxidoreductases*;
(b) transferases;
(c) hydrolases;
(d) lyases;
(e) isomerases;
(f) ligases;
(g) combinations of any of (a)-(f).

11. The composition of claim 10 (c) wherein the hydrolase is selected from:

(a) ester hydrolases;
(b) glycosyl hydrolases;
(c) ether (e.g. thioether) hydrolases;
(d) peptide hydrolases;
(e) combinations of any of (a)-(d).

12. The composition of claim 11 (a) wherein the ester hydrolase is:

(a) a carboxylic ester hydrolase (e.g. a lipase);
(b) a thioester hydrolase;
(c) a phosphoric monoester hydrolase;
(d) a phosphoric diester hydrolase;
(e) a triphosphoric monoester hydrolase;
(f) a sulphuric ester hydrolase;
(g) a diphosphoric monoester hydrolase;
(h) combinations of any of (a)-(g).

13. The composition of claim 11 (b) wherein the glycosyl hydrolase hydrolyses *O*-glycosyl (e.g. being any of α-glucosidase, ß-glucosidase, α-galactosidase and ß-galactosidase), *N*-glycosyl or *S*-glycosyl compounds (e.g. being myrosinase).

14. The composition of claim 11 (d) wherein the peptide hydrolase is selected from:

(a) α-aminoacylpeptide hydrolases;
(b) peptidylamino-acid or acylamino-acid hydrolases;
(c) dipeptide hydrolases;
(d) dipeptidylpeptide hydrolases;
(e) peptidyldipeptide hydrolases;
(f) proteinases (e.g. serine proteinases, SH-proteinases, acid proteinases or metalloproteinases);
(g) combinations of any of (a)-(f).

15. The composition of any one of the preceding claims wherein the microemulsion droplets and/or hydrated reverse micelles are food grade.

16. The composition of claim 15 wherein the oil is animal or vegetable oil.

17. The composition of claim 16 wherein the vegetable oil is sunflower oil.

18. The composition of any one of claims 1-17 wherein the droplets or micelles comprise a surfactant.

19. The composition of claim 18 wherein the surfactant has a low hydrophilic-lipophilic balance.

20. The composition of claim 18 or claim 19 wherein the surfactant is selected from:

(a) a sorbitan ester (e.g. sorbitan monooleate);
(b) a glycerol derivative (e.g. stearyl monoglyceride);
(c) a phospholipid (e.g. lecithin);
(d) naturally occurring phosphoglycerides (e.g. phosphatidylcholine or phosphatidylethanolamine)
(e) mixtures of any of (a)-(d) (e.g. a mixture of lecithin and sorbitan monooleate).

21. The composition of any one of claims 18-20 further comprising a co-surfactant.

22. The composition of claim 21 wherein the co-surfactant is selected from:

(a) a short chain alcohol (e.g. ethanol, propanol or butanol);
(b) propylene glycol;
(c) mixtures thereof.

23. The composition of any one of claims 18-22 wherein the packing parameter $S_p$ of the surfactant is greater than 1, wherein:

$$S_p = V(al)^{-1}$$

where $V$ is the effective packing volume of the surfactant hydrocarbon tail, $l$ is the length of the hydrocarbon tail and $a$ is the effective headgroup area at the oil-water interface.

24. The composition of any one of claims 18-23 wherein the surfactant and/or co-surfactant is a double tail and/or medium tail length surfactant.

25. The composition of any one of claims 18-24 wherein the molecular geometry of the surfactant and/or co-surfactant tail(s) is selected such that $V$ is increased relative to $a$.

26. The composition of any one of the preceding claims wherein the $R$ value of the microemulsion droplets and/or hydrated reverse micelles is less than 10 (e.g. less than 5).

27. The composition of any one of the preceding claims wherein the droplet/micelle size is 1-100 nm (e.g. 1-10 nm).

28. The composition of any one of the preceding claims comprising a microemulsion having the composition:

(a) about 80% wt vegetable oil (e.g. sunflower oil);
(b) about 15% wt lecithin and sorbitan monooleate surfactant mix;
(c) about 2 to about 4% wt ethanol co-surfactant;
(d) about 0.5 to about 3.0% wt water;
(e) about 1% wt flavour precursor;
(f) about 0.5% wt enzyme.

29. A composition comprising food-grade water-in-oil microemulsion droplets or hydrated reverse micelles (e.g. having and $R$ value of less than 10 (e.g. less than 5) and/or a droplet/micelle size of 1-100 nm (e.g. 1-10 nm).

30. The composition of claim 29 wherein:

(a) the oil is as defined in claim 16 or claim 17; and/or
(b) the droplets or micelles comprise a surfactant or surfactant/co-surfactant (for example as defined in any one of claims 18-25); and/or
(c) the composition comprises a microemulsion having the composition as defined in claim 28; and/or
(d) the droplet/micelle cores have low water activity and contain a latent flavour system as defined in any one of claims 3 to 14.

31. The composition of any one of the preceding claims wherein the microemulsion is in the form of an organogel (e.g. a gelatin-based organogel).

32. A process for the organic synthesis of a flavour, fragrance, flavour precursor or fragrance precursor comprising the step of enzymically synthesising the flavour, fragrance or precursor in a water-in-oil microemulsion.

33. The process of claim 32 wherein the microemulsion droplet cores contain:

(a) a substrate; and

(b) an enzyme which acts on the substrate to produce the active flavour, fragrance or precursor.

34. The process of claim 32 or 33 wherein the enzymic synthesis is controlled by varying the *R* value of the microemulsion to alter the catalytic activity and/or specificity of the enzyme.

35. The process of claim 34 wherein the enzyme is a hydrolase and the *R* value is selected to shift the equilibrium position of the enzymic reaction such that the enzyme acts synthetically rather than hydrolytically, for example to synthesise any of:

(a) an ester;

(b) a glycosyl compound;

(c) an ether (e.g. a thioether);

(d) a peptide;

(e) a carbon-nitrogen bond (e.g. in an amide, amidine or nitrile compound);

(f) an acid anhydride (e.g. a phosphoryl- or sulphonyl-containing anhydride);

(g) a carbon-carbon bond (e.g. in a ketone compound); or

(h) a halide, phosphorous-nitrogen, sulphur-nitrogen or carbon-phosphorous bond.

36. The process of any one of claims 32 to 35 wherein the active flavour, fragrance or precursor is recovered by driving the microemulsion dropelts through a phase boundary by subjecting the microemulsion droplets to a temperature change to yield a Winsor I, Winsor II or Winsor III type microemulsion system.

37. An active flavour, fragrance, flavour precursor or fragrance precursor obtainable by (or produced by) the process of any one of claims 32 to 36.

38. A system for use in the process of any one of claims 32 to 36, the system comprising a water-in-oil microemulsion, wherein the microemulsion droplet cores contain:

(a) a substrate; and

(b) an enzyme which acts on the substrate to produce the active flavour, fragrance or precursor.

39. The system of claim 38 wherein:

(a) the oil is as defined in claim 16 or claim 17; and/or

(b) the droplets or micelles comprise a surfactant or surfactant/co-surfactant (for example as defined in any one of claims 18-25); and/or

(c) the composition comprises a microemulsion having the composition as defined in claim 28; and/or

(d) the microemulsion is in the form of an organogel (e.g. a gelatin-based organogel).

40. A reactor comprising the system of claim 38 or claim 39.

41. A foodstuff comprising the flavour releasing composition of any one of claims 1 to 28.

42. The foodstuff of claim 41 which is:

(a) a babyfood; or

(b) a bakery product (for example a bread, tart, pie, yeasted goods or a cake); or

(c) a bakery supply product (for example, a custard or a bakery filling or topping); or

(d) a batter; or

(e) a breading; or

(f) a cereal; or

(g) a confectionery; or

(h) a flavour or beverage emulsion; or

(i) a fruit filling; or

(j) a gravy, soup, sauce or food thickener;

(k) a frozen, chilled or ambient stable gravy, soup, sauce or food thickener; or

(l) a pasteurised, retorted or UHT treated gravy, soup, sauce or food thickener; or

(m) a meal or meal component; or

(n) a meat product; or

(o) a petfood; or

(p) a potato product; or

(r) a dairy product (e.g. a dessert or yogurt); or

(s) a salad dressing; or

(t) a snack or cracker; or

(u) a spread; or

(v) a pasta product (e.g. a noodle); or

(w) a tart or pie (e.g. a fruit tart or pie);
(x) a beverage (e.g. a sports drink or fruit juice drink).

43. Use of a composition comprising water-in-oil microemulsion droplets and/or hydrated reverse micelles (e.g. a food grade composition as defined in any one of claims 29 to 31) for flavour delivery (e.g. flavour delivery in the mouth).

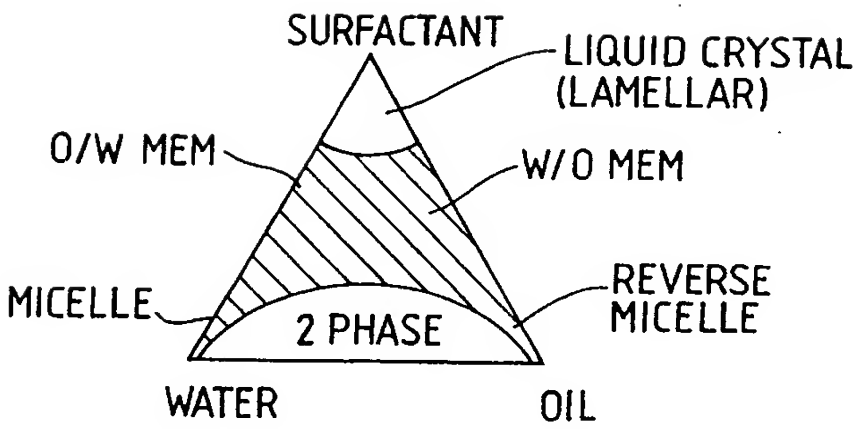


Fig.1. Shaded portion indicates single phase MEM region.

# INTERNATIONAL SEARCH REPORT

Intern· ·nal Application No
PCT/GB 99/01659

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passage | Relevant to claim No. |
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| X | US 5 045 337 A (EL-NOKALY MAGDA ET AL) 3 September 1991 (1991-09-03) column 2, line 50 - line 65 column 8, line 6 - line 61 --- | 1-43 |
| X | ENZYME AND MICROBIAL TECHNOLOGY, vol. 21, no. 2, - 1997 pages 117-123, XP002119757 | 1-43 |
| Y | page 117 --- | 1-43 |
| X | JOURNAL OF FERMENTATION AND BIOENGINEERING, vol. 76, no. 2, - 1993 pages 98-104, XP002119758 page 98 --- | 1-43 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 21 October 1999 | 05/11/1999 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Bendl, E |

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## INTERNATIONAL SEARCH REPORT

Intern· ·nal Application No
PCT/GB 99/01659

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ' | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
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| Y | WO 96 23425 A (NESTLE SA) 8 August 1996 (1996-08-08) page 3 -page 4 ----- | 1-43 |

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...formation on patent family members

Interr ...nal Application No
PCT/GB 99/01659

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